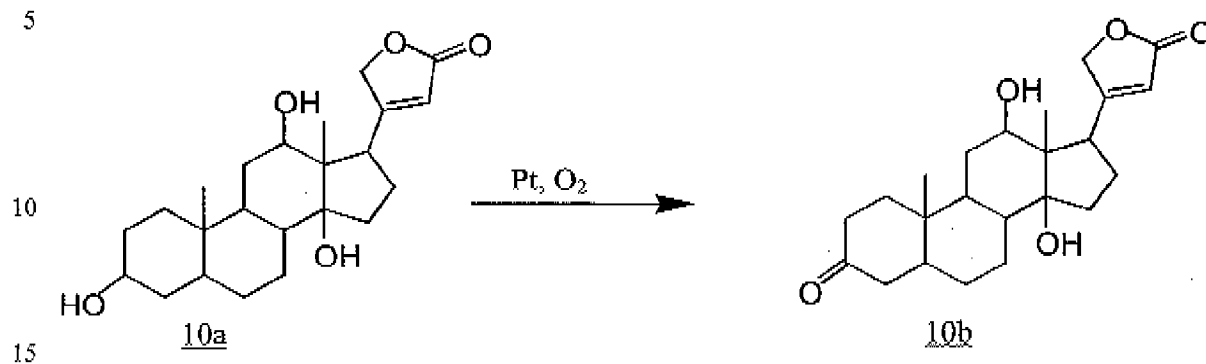
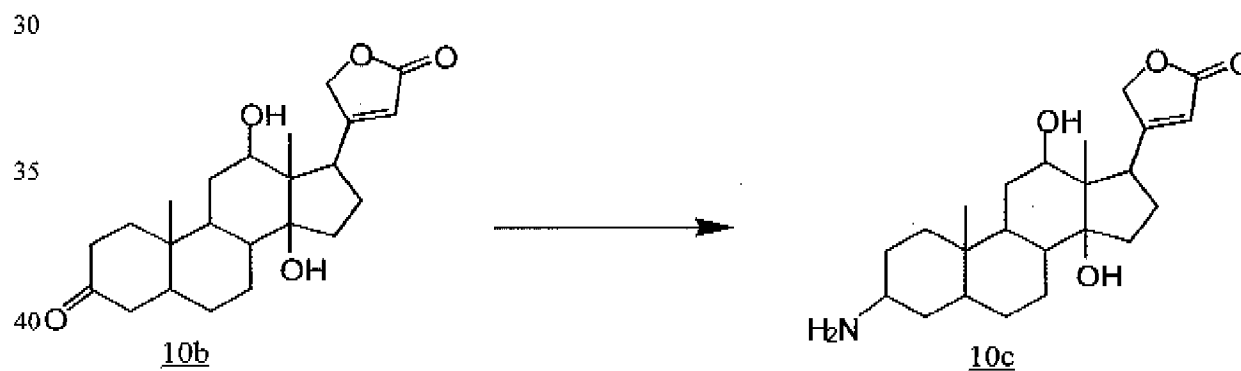


REPLACE pages 70-74 of the specification with the following pages:

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4. Preparation of Boc-Linked-Dig (10)**a. 3-ketodigoxigenin (10b)**

Platinum oxide (489 mg) was suspended in 20 ml of deionized water and reduced to platinum metal in a Parr hydrogenation apparatus at 40 psi of hydrogen at room temperature for two hours. The platinum was then transferred to a solution of 500 mg (1.28 mmol) of digoxigenin 10a (prepared as described in Ferland, J.M. *Can. J. Chem.* 1974, 652) in 50 ml of dry acetone. The reaction was placed under a steady stream of air and stirred at room temperature for three days. The reaction was worked up by filtering the catalyst off through a pad of Celite and evaporating the solvent. 449 mg (90% yield) of the crude product 10a was obtained, which was of sufficient purity for use in the subsequent step.

b. 3-Aminodigoxigenin (10c)

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To 448 mg (1.26 mmol) of 3-ketodigoxigenin **10b** dissolved in 5 ml of methanol was added 100 mg (6.74 mmol) of ammonium acetate. The reaction was stirred at room temperature under argon for 30 minutes. Then 85 mg (1.35 mmol) of sodium cyanohydride was added and stirring was continued for two more hours at room temperature.

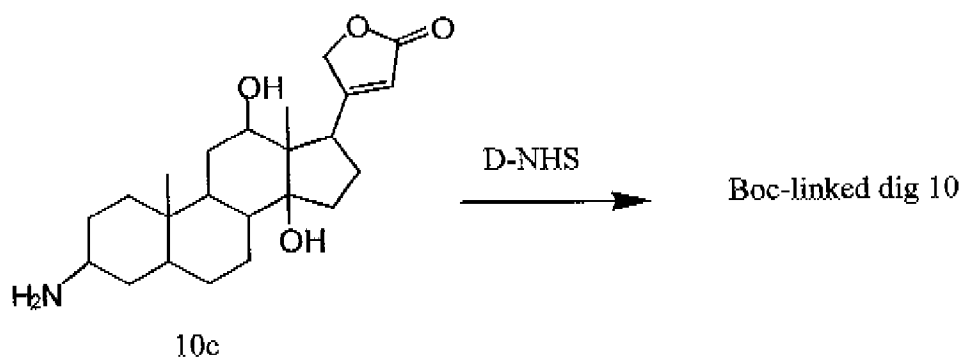
- 5 The 3-aminodigoxigenin **10c** was formed rapidly. The reaction was quenched by addition of 4 ml of glacial acetic acid and evaporated to dryness. The crude product was dissolved in 20 ml of n-butanol, then extracted with 25 ml of a solution of 1g/ml of potassium carbonate in water. The aqueous phase was washed with 3x25 ml of n-butanol. The combined organic phase was dried and evaporated to dryness to yield 365 mg (69%) of the
- 10 **10c** as white solid. The crude product was of sufficient purity for use in the next step. NMR: (CD₃OD) 0.79(s,3H,C18), 0.96(s,3H,C19), 1.00(s, 3H, C19), 1.1-2.3 (m, CH₂'s and CH's), 3.4 (m, 1H, C12), 4.95(m, 2H, C21), 5.91(s, 1H, C22). M.S. : (EI) 473 M+

d. Boc-linked dig 10

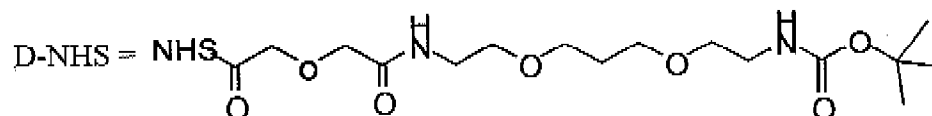
15

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25



30



10d

35

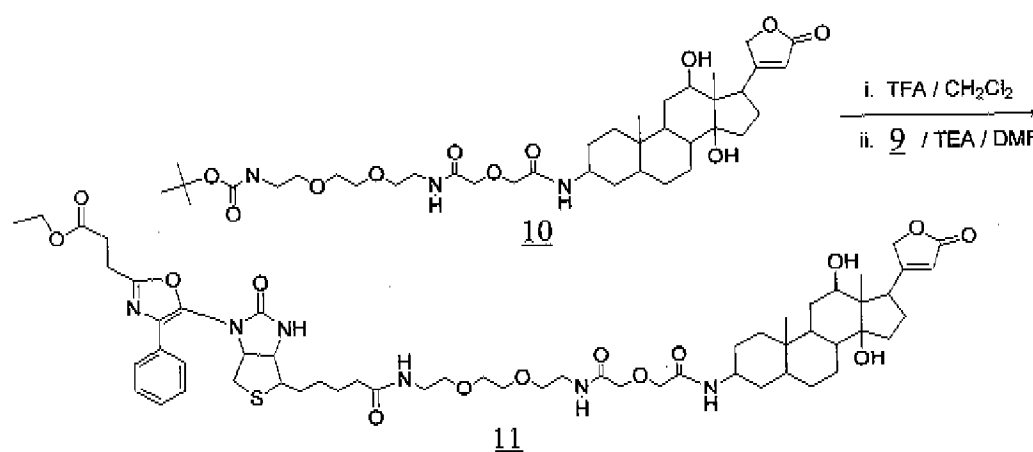
To a solution of N-tboc acid **10c** (Aldrich, 721 mg, 2.0 mmol) in THF (25 ml) were added DCC (412 mg, 2.0 mmol) and NHS (287 mg, 2.5 mmol). The mixture was stirred

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overnight. The reaction was then filtered through glass wool and added to a stirred solution of aminodigoxigenin **10c** and triethyl amin (101 mg) in 25 ml of dichloromethane. The reaction was stirred overnight and then was concentrated under vacuum. Dichloromethane (50 ml) was then added and extracted with 3x50 ml of 0.5 N HCl and finally with water (100ml). The organic phase was then dried and evaporated to 1/3 of the volume and applied to ten 20x20 silicagel GF plates (CH₃OH:H₂O, 1:9), to give 1.2 g of the pure product.

5. Preparation of Digoxigenin-Linked-Biotin-Oxazole-R-CO₂Et (**11**).

10

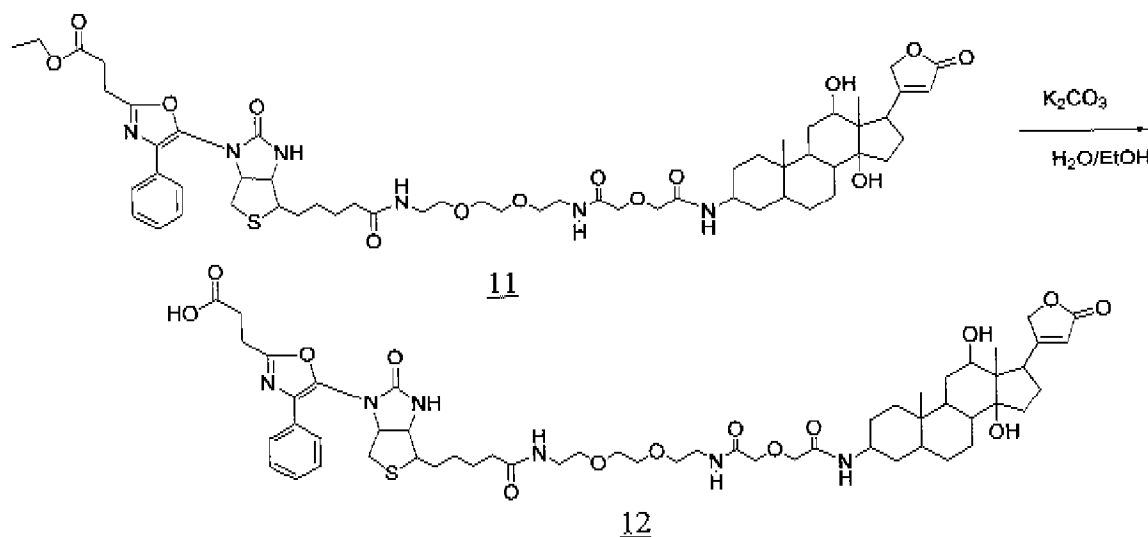


Boc-Linked-Dig (**10**) (30 mg, 0.04 mmol) was deprotected by stirring in 1.0 ml (1:5) TFA-CH₂Cl₂ for 15 min at ambient temperature. The reaction mixture was concentrated and the volatiles removed by dissolving the residue in CH₂Cl₂ and concentrating a second time. The residue was redissolved in 2 mL CH₂Cl₂ and neutralized with TEA in 1 mL DMF. The NHS-Biotin-Oxazole-R-CO₂Et filtrate (**9**) was rinsed with 0.5 mL THF directly into the DMF diluted amino-Linked-Dig solution. The solution was bubbled with argon and stirred for 2h at ambient temperature. TLC (SiO₂ : 10% MeOH/CH₂Cl₂) confirmed that the reaction had gone to completion. The crude reaction mixture was applied directly to a pre-equilibrated (5% MeOH/CH₂Cl₂) preparative silica gel plate and eluted with 15% MeOH/CH₂Cl₂. The pure product fractions were combined affording 16 mg DLBOR-CO₂Et (**11**). TLC (SiO₂ : 0.1% HOAc, 15% MeOH/CH₂Cl₂, single spot R_f 0.3). 1H-

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NMR (250MHz, D₂O) δ : 7.9 – 7.1 (m, 5H), 5.7 (s, 1H), 5.65 (s, 1H), 4.9 (s, 2H), 4.6 (7H + H₂O), 4.25 (m, 2H), 4.1 (m, 2H), 3.95 (m, 2H), 3.65 (m, 2H), 3.6 – 3.25 (m, 10H), 3.25 – 2.85 (m, 7H), 2.85 (m, 2H), 2.25 (m, 1H), 2.0 (m, 2H), 1.75 (m, 2H), 1.6 – 0.9 (m, 26H), 0.75 (s, 3H), 0.60 (s, 3H).

5 **6. Preparation of Dig-Linked-Biotin-Oxazole-R acid (DLBOR-CO₂H) (12).**



10 To 10.0 mg DLBOR-CO₂Et (**11**) (9.0 μ mol), dissolved in 100 μ L EtOH, was added 1.0 mL of 25 mM K₂CO₃ in 5% H₂O/EtOH. The solution was stirred under argon overnight at ambient temperature. The reaction was followed by TLC (SiO₂ : 5% MeOH/CH₂Cl₂), after 18h the pH of the reaction was adjusted to 3-4 with 0.1 N HCl. TLC (C18 : 95% CH₃CN/H₂O, major spot R_f 0.5, and SiO₂ : 0.1% HOAc, 15% MeOH/CH₂Cl₂, major spot

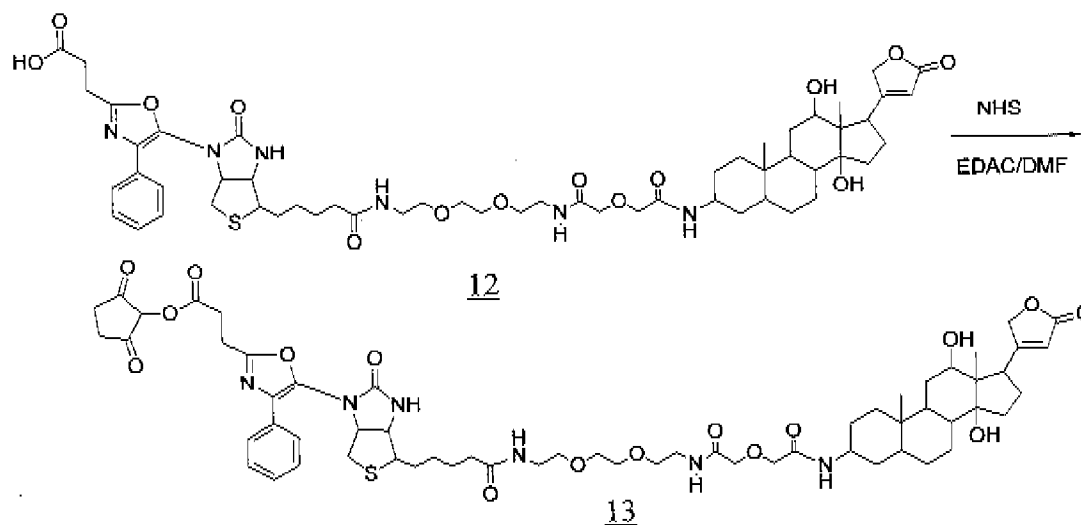
15 R_f 0.65, and a minor spot R_f 0.90 (the minor spot appeared to be digoxin-linked biotin), NMR confirmed the complete hydrolysis of the ethyl ester. Dig-Linked-Biotin (DLB) release by ¹O₂ was confirmed by the soluble sensitizer method (see Example 5). The solvent was removed under vacuum and a 2.0 mg (1.7 μ mol) portion of the crude DLBOR acid (**12**) was redissolved in 0.50 mL DMF for use in the next step.

20

7. Preparation of Dig-Linked-Biotin-Oxazole-R-NHS ester (DLBOR-CO₂NHS)

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ester) (13).



- 5 To 2.0 mg (1.7 μmol) DLBOR-CO₂H (**12**) in 0.5 mL DMF was added 10 μL of NHS (0.23 mg, 2.0 μmol)/DMF and EDAC. The solution was stirred under argon overnight at ambient temperature. The solvent was removed under vacuum and the residue was redissolved in 100 μL of degassed DMF. This DMF solution of DLBOR-CO₂NHS (**13**) was attached directly to beads in the same manner as described for DLBAR-CO₂NHS (see
- 10 **Figure 3** and Example 2).